Persistent Organic Pollutants in Norwegian Men from 1979 to 2007: Intraindividual Changes, Age–Period–Cohort Effects, and Model Predictions

Therese Haugdahl Nøst, Knut Breivik, OleMartin Fuskevåg, Evert Nieboer, Jon Øyvind Odland and Torkjel Manning Sandanger

Persistent Organic Pollutants in Norwegian Men from 1979 to 2007: Intraindividual Changes, Age–Period–Cohort Effects, and Model Predictions

Therese Haugdahl Nøst,[1,2,3] Knut Breivik,[4,5] Ole-Martin Fuskevåg,[3] Evert Nieboer,[1,6] Jon Øyvind Odland,[1] and Torkjel Manning Sandanger[1,2]

[1]Department of Community Medicine, University of Tromsø, Tromsø, Norway;

[2]NILU-Norwegian Institute for Air Research, Fram Centre, Tromsø, Norway;

[3]University Hospital of North Norway, Tromsø, Norway;

[4]NILU-Norwegian Institute for Air Research, Kjeller, Norway;

[5]Department of Chemistry, University of Oslo, Oslo, Norway;

[6]Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada.

Corresponding author: Therese Haugdahl Nøst, NILU-Norwegian Institute for Air Research, Fram Centre, NO-9296 Tromsø, Norway. Tel.(+47)77750398. zhn@nilu.no.

Running title: Intraindividual changes in POPs: 1979-2007

Competing Interests: The authors declare they have no competing financial interests.

1

Abstract

Background. Longitudinal monitoring studies of persistent organic pollutants (POPs) in human populations are important to better understand changes with time and age, and for future predictions.

Objectives. To describe blood POP time trends on an individual level, investigate age-period-cohort effects, and compare predicted polychlorinated biphenyl (PCB) concentrations to measured values.

Methods. Serum was sampled in 1979, 1986, 1994, 2001, and 2007 from a cohort of 53 men in Northern Norway, and analyzed for 41 POPs. Calendar year (period), age, and birth cohort effects were assessed by graphical analyses and mixed effect models. Predicted concentrations of four PCBs for each sampling year were derived using the CoZMoMAN model.

Results. The median decreases in summed serum POP concentrations (lipid-adjusted) in 1986, 1994, 2001, and 2007 relative to 1979 were -22%, -52%, -54%, and -68%, respectively. Substantial declines were observed in all POP groups with the exception of chlordanes. Time period (reflected by sampling year) was the strongest descriptor of changes in PCB-153 concentrations. Predicted PCB-153 concentrations were consistent with measured concentrations in the study population.

Conclusions. Results suggest substantial intraindividual declines in serum concentrations of legacy POPs from 1979 to 2007 in men from Northern Norway. These changes are consistent with reduced environmental exposure during these 30 years, and highlight the relation between historic emissions and POP concentrations measured in humans. Observed data and interpretations are supported by estimates from the CoZMoMAN emission-based model. A longitudinal decrease in concentrations with age was evident for all birth cohorts. Overall,

findings support the relevance of age-period-cohort effects to human biomonitoring of environmental contaminants.

Introduction

The use of persistent organic pollutants (POPs) in agriculture and industry increased markedly beginning in the 1930s (AMAP 2004). As concerns for detrimental effects on the environment and human health increased, measures to reduce or eliminate the production and use of POPs were initiated in many countries from the 1970s onwards (AMAP 1998). Consequently, global emissions of legacy POPs have largely followed the same trends. Reduced use and emissions of legacy POPs were followed by declining POP concentrations in air and biota (e.g. Hung et al. 2010; Rigét et al. 2010), which has led to reduced human exposure. Accordingly, declining concentrations of most banned compounds have been reported in the few available human longitudinal POP studies (Hagmar et al. 2006; Hovinga et al. 1992; Høyer et al. 2000; Tee et al. 2003; Vo et al. 2008).

POP concentrations have frequently been reported to be positively associated with age (Hardell et al. 2010; Rylander et al. 1997; Wolff et al. 2005) and to birth cohorts (Bjerregaard et al. 2001; Perry et al. 2005; Wolff et al. 2007) in human cross-sectional studies. In such studies, age and birth cohort effects are confounded (Glenn 2003; Quinn and Wania 2012). In longitudinal epidemiologic studies, age effects reflect differences in risk factors between age groups, period effects reflect temporal changes in factors that affect all individuals in a population, and birth cohort effects reflect generation-specific influences (Glenn 2003; Holford 1991; Palmore 1978). The interdependence of age, period, and cohort effects produces mutual confounding in time-trend studies. This has previously not been considered in empirical studies of POP time trends.

A person's lifetime environmental exposure to any POP (i.e., the intensity and duration of their environmental exposure) depends on when they were born relative to the time of peak environmental concentrations. Increasing age is associated with physiological changes (e.g. changes in body composition and metabolism) and changes in dietary patterns that may influence both the intake and the elimination of POPs. Environmental exposures and dietary habits also differ among birth cohorts, and contribute to differences in the duration and intensity of exposures to specific POPs. Finally, periodic patterns reflect changes in environmental POP concentrations related to historic emissions and environmental persistence, in addition to temporal trends in dietary intakes.

The present study is based on 5 repeated measurements of serum POPs concentrations during 1979-2007 in a cohort of 53 men from Northern Norway. Our primary aims were to describe intraindividual changes in POP concentrations and composition, and investigate age-period-cohort effects. In addition, we compared measured POPs concentrations and observed age-period-cohort patterns to predictions based on the time-variant CoZMoMAN model (Breivik et al. 2010). Use of emission-based mechanistic modeling in combination with the unique empirical data available for the study population provides insight into the relationship between environmental emissions and observed concentrations in humans.

Subjects and methodology

Study population and subject selection

Five repeated population surveys in the Tromsø study (summarized in Jacobsen et al. 2012) took place in the municipality of Tromsø, Northern Norway in 1979, 1986-1987 (hereafter only referred to as 1986), 1994-1995 (1994), 2001, and 2007-2008 (2007). Of 60 randomly selected

men, 53 had sufficient sample volumes in ≥3 sampling years (11 missing samples were randomly distributed across sampling years). In total, the present analyses comprised 254 blood samples from 53 men. Birth year and body mass index information was extracted from questionnaires. The study was approved by the Regional Committees for Medical Research Ethics. Participation was voluntary and all participants provided informed consent. Blood samples were stored at -70 °C until analysis.

Analytical methodology

All contaminant analyses were performed during 2011 at the laboratories of the Norwegian Institute for Air Research (NILU) and the University Hospital of Northern Norway (UNN) in Tromsø, Norway. Serum samples were extracted and analyzed for PCBs and organochlorine pesticides [chlordanes, hexachlorohexanes (HCHs), hexachlorobenzene (HCB), 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (p,p'-DDT) and its metabolites (DDTs), and toxaphenes]. A complete list of the individual POPs is provided in Supplemental Material, Table S1.

Extraction and clean up. Methods were modified from Sandanger et al. (2003; 2007). Serum (average of 0.92 g and range: 0.24-1.12 g) was weighed and 26 ^{13}C-labeled internal standards, deionized water saturated with ammonium sulphate (1 ml), methanol (2 ml), and hexane (6 ml) were added. The tube was vortexed and shaken for 1 hr. Samples were centrifuged (1,200 rpm, 6 min) in an Eppendorf 5702R centrifuge (Eppendorf, Hamburg, Germany), and the supernatant hexane was pipetted off. The extraction protocol was repeated with 6 ml of hexane, and the hexane supernatants were combined and evaporated to 0.5 ml in a heated vacuum evaporation unit. Subsequent clean up using SPE columns (Florisil, 1 g, deactivated) was performed using an automated liquid handling system. The column was pre-washed with hexane/DCM (12 ml;

9:1w/w) and hexane (12 ml) before the extract was applied. Analytes were subsequently eluted with hexane/DCM (12 ml; 9:1w/w), evaporated to 0.2 ml, transferred to a GC-vial, further reduced to ~30 µl by gentle nitrogen flow, and recovery standard (octachloronaphtalene) was then added.

Instrumental analysis. Chlorinated pesticides (excluding DDTs) (1µl injection volume) were analyzed on an Agilent 7890A gas chromatograph (GC) equipped with a 5975c mass spectrometer (MS) (instrumental details are described in Hansen et al. 2010). The MS operated in selected ion monitoring (SIM) and negative chemical ionization (NCI) modes at 160 °C. All POPs were analyzed with the GC temperature program used in Hansen et al. (2010), with the exception of the toxaphenes, which were analyzed separately with the following conditions: 70 °C (3 min), 25 °C min^{-1} to 180 °C (0 min), and 15 °C min^{-1} to 280 °C (5 min).

PCBs and DDTs were analyzed on the same GC as described above but with a Quattro Micro triple quadrupole MS (Waters Corporation, Manchester, UK). Injector settings, GC column, carrier gas, and the temperature program were the same as described for pesticide analyses. The MS operated in MS/MS (MRM) mode with an EI source at 220°C. Collision gas was argon (~0.23 Pa). Information regarding ion transitions has been published previously (Pitarch et al. 2007).

Lipid determination. Analyses of triglycerides, phospholipids, free cholesterol, and total cholesterol were determined enzymatically by Unilab Analyse AS, Tromsø, Norway, and a summed lipid concentration was calculated according to the equation proposed by Akins et al. (1989).

Quality assurance and sample integrity

Quality control in POP and lipid analyses. To assess laboratory-derived sample contamination and method accuracy and reproducibility, blanks (n = 9) and standard reference materials (SRMs) [SRM® 1958 (n = 9) and 1957 (n = 9), both from the National Institute of Standards and Technology, Gaithersburg, MD, USA] were processed along with samples. Results for SRMs indicated analytical uncertainties within +/-20% of assigned values (within +/- 5% for many compounds). The NILU laboratory routinely participates in the international AMAP Ring Test for Persistent Organic Pollutants in Human Serum, and have performed well (within +/-20% of assigned values). Concomitantly, summed lipid concentrations in the test samples (n = 10) were within 15% deviation from assigned values. [Ring test results are available from http://www.inspq.qc.ca/ctq/paqe/amap/rapports.asp?Page=2c&partie=2&Lg=en&PCI=6.]

Average recoveries of internal standards were 81%, 83%, 77%, 79%, and 56% for the 1979, 1986, 1994, 2001, and 2007 samples, respectively. The internal standard recovery for the 2007 samples was low in one sample preparation batch (53% of 2007 samples); however, there was no association between recoveries and concentrations (data not shown). Recoveries were below 30% in three samples, but they were not excluded from statistical analyses because they did not constitute extreme observations nor deviated in model diagnostic plots. When isotopic mass ratios deviated by more than 20% from the quantification standards, the results were rejected. PCBs 138/163 and PCBs 28/31 coeluted, and concentrations were summed for analyses. The limits of detection (LODs) were software-generated and corresponded to signal-to-noise ratios of 3. Consistent amounts of *β* –HCH and *oxy*-chlordane were measured in blanks, and average blank concentrations for these compounds were therefore subtracted from all samples.

Estimation of desiccation and lipid degradation. To correct for spuriously high POP concentrations caused by evaporation during long-term storage, serum sodium (Na^+) was measured and used to adjust lipid and POP concentrations. In samples with Na^+ concentrations >165 nmol/l (3% of samples), lipid and POP concentrations were adjusted by the ratio $[Na^+]_{mean}/[Na^+]_{sample}$ (Krieger et al. 1994). Na^+ determinations were conducted at UNN, Tromsø, Norway using an ion selective electrode method.

Total cholesterol and triglycerides were measured previously and, after adjusting current results for desiccation, the past and current measurements deviated less than 10% and correlations between the two measurements increased (data not shown).

Time-variant model simulations of PCBs in blood

Simulations of lipid-normalized serum concentrations of PCB 118, 138, 153, and 180 for 1979, 1986, 1994, 2001, and 2007 were carried out using the time-variant multimedia mechanistic CoZMoMAN model (Breivik et al. 2010). Previous CoZMoMAN model predictions for PCB concentrations and their temporal changes in women were within the ranges of measured concentrations (Breivik et al. 2010; Quinn and Wania 2012). Simulations were performed assuming time-variant emission scenarios (Quinn et al. 2011). In general, model parameters were set as outlined in Breivik et al. (2010). Specifically, we assumed that trends and concentrations in the environment and food-chains in Northern Norway are similar to the trends for Sweden and parts of Southeastern Norway that were used for model development, except for the dietary input parameters. We assumed that fish consumption in the population of older men from Northern Norway was higher than the original model input, and therefore derived separate predictions based on average Norwegian fish consumption and three categories of higher fish consumption

(for detailed information, see Supplemental Material, page 3-4 and Table S2). Model predictions for PCB-153 for the birth years 1930, 1935, 1940 and 1945 (within the range of birth years of the study subjects) were obtained assuming that all of the birth cohorts had equal fish consumption, or that fish consumption differed among birth cohorts, with the earliest birth cohort consuming the most fish, and the latest cohort the least.

Data treatment and statistical methods

Statistical analyses were executed using R, ver.2.13.1. Statistical significance was defined as $p < 0.05$. All POP results were lipid-adjusted and $\log_e$-transformed in the statistical analyses. Three samples from 1979 with high (>Median+2*SD) and three samples from 1994 with low lipid-adjusted concentrations (<Median-2*SD) were excluded from statistical models to obtain the most appropriate model estimates, but analyses including these samples and when performed on wet weight concentrations gave the same main results (data not shown). Lipid concentrations were missing for one sample, and thus the numbers of observations in the statistical analyses were 51, 51, 45, 48, and 52 for the five time points.

Summed POP concentrations were calculated based on lipid-adjusted concentrations of compounds with > 60% detection and for values below LOD, the individual concentration estimates were used. Summary statistics for compounds with detection frequencies between 20% and 80% were calculated for each sampling year using the Kaplan-Meier method with the NADA package for R according to Helsel (2005).

Spearman`s ρ values were calculated for correlations. The Wilcoxon signed rank test was used to test differences in POP concentrations between sampling years, and the Kruskal-Wallis rank sum

test was used to test differences between birth year groups (categorized according to quartiles) in each sampling year.

Age–period–cohort (APC) effects were assessed for serum concentrations of PCB-153 using age and birth cohort groups categorized according to quartiles. Mixed effect models (lme4 package for R) that included a random slope for sampling year and subject-specific random terms (to allow subject-specific random variation) were used to estimate periodic changes in PCB-153 concentrations and potential age-specific and birth cohort-specific effects. Despite collinearity, models used to assess APC effects must include all three time parameters (Palmore 1978), therefore, we used mixed effect models with two parameters modeled as fixed effects and the third modeled as a random effect (Ding et al. 2007). Body mass index was also assessed as a fixed effect. Confidence intervals for coefficients were obtained post hoc (glht in multcomp R package). Akaike's information criterion (AIC) was used to compare models. The non-parametric Friedman`s test, was used to test differences across all measurements.

A graphical examination of age–period–cohort effects for PCB-153 was carried out by plotting all six combinations of the three time factors according to Ahacic et al. (2012) to assess longitudinal patterns (i.e., concentrations according to birth cohort and sampling period or age), time-lag patterns (concentrations according to age and sampling period or birth cohort), or cross-sectional patterns (concentrations according to sampling period and age or birth cohort).

Results

Characteristics of study participants

Median ages at the first and last sampling were 43 and 71, respectively (Table 1). The median birth year was 1936 (range: 1925 to 1950). The number of subjects in each age and birth cohort quartile is included in Supplemental Material, Table S3.

Intra-individual changes in POP concentrations

Serum POP concentrations in each sampling year are presented for selected compounds in Figure 1 (see Supplemental Material, Table S4 for complete data for all analyzed POPs). The median individual decreases in summed POP concentrations in 1986, 1994, 2001, and 2007 relative to the median concentration in 1979 were -22%, -52%, -54%, and -68%, respectively. Substantial declines were observed for all POPs with the exception of chlordanes (e.g., *t*-Nonachlor in Figure 1). Overall, decreases were observed from 1979 in concentrations of HCHs, HCB, c-chlordane, DDTs, and most penta-chlorinated PCBs (PCB 99, 101, 105, 118, and 123) and hexa-chlorinated PCBs (PCB 128, 141, 149, 153, and 167). Declining trends of many POPs were exponential, especially for *p,p'*-DDT (R^2=0.78 for fitted exponential trend line, data not shown). Concentrations of chlordanes (except *c*-chlordane), Mirex, toxaphenes, and ≥hepta-chlorinated PCBs (PCB 170, 180, 187, and 194) initially increased from 1979 to 1986, and declined in subsequent years. Concentration differences across all sampling years were significant for all POPs (Friedman`s test, p <0.001), however the absolute differences in the concentrations of chlordanes were small (e.g., 47 and 45 ng/g lipid-adjusted for t-Nonachlor in 1979 and 2007, respectively). Within individuals, the concentrations of most POPs were higher in 1979 than in

2007, but time trends varied among individuals, as shown for PCB-153 (Figure 2; individuals grouped according to birth year).

Relative contributions of individual POPs to summed POP concentrations (as %) are shown in Figure 3. Clearly, *p,p'*-DDE; PCBs 153, 138/163, and 180; and HCB were the most prominent (67-73% of sum in the different sampling years). *p,p'*-DDE/*p,p'*-DDT ratios were 12, 20, 29, 41, and 55, for 1979, 1986, 1994, 2001 and 2007, respectively.

POP correlations across sampling years

The correlations of concentrations (lipid-adjusted) in any pair of consecutive sampling years increased across the study period for most compounds. Concentrations correlated significantly between 1986-1994, 1994-2001 and 2001-2007 for PCB-153 ($\rho = 0.72$, 0.80, 0.87, respectively, all $p < 0.001$), and between all sampling years for *p,p'*-DDE ($\rho = 0.44$, 0.79, 0.87, 0.89 between 1979-1986, 1986-1994, 1994-2001 and 2001-2007, respectively, all $p < 0.002$).

Inter-compound correlations

In 1979, correlations were strong ($\rho > 0.85$) between PCB-153 and *oxy*-chlordane, Mirex, and PCB 99, 118, 138/163, 156, 157, 167, 170, 180, 183, 187, 189, and 194. The correlations between PCB-153 and other POPs were also strong in the subsequent sampling years ($\rho > 0.85$; n= 13, 8, 10 and 9 compounds for 1986, 1994, 2001, and 2007, respectively), yet slightly weaker over time.

Predicted PCB concentrations

Measured and predicted concentrations (ng/g lipid) of PCB 118, 138, 153, and 180 from CoZMoMAN simulations for a 1935 birth cohort of men are presented in Supplemental Material,

Figure S1 for the five sampling years according to four different assumptions regarding fish consumption. Model predictions were generally consistent with measured concentrations, especially for PCB 153 and 180, but overestimated concentrations of PCB-118 and underestimated concentrations of PCB-138. Predicted trends (assuming equal fish consumption among birth cohorts) for PCB-153 concentrations for men born in 1930, 1940, and 1945 (Supplemental Material, Figure S2) showed similar trends among birth years.

Estimated age-period-cohort effects on changes in PCB-153 concentrations

Estimates from mixed effect models of PCB-153 concentrations in the different sampling years, with age, period, and birth cohort modeled as fixed predictors or as random effects (accounting for subject-specific variation) are shown in Table 2. The best fitting model included period and birth cohort as predictors and age as a random effect. Estimates for the period effects (i.e., changes according to sampling year) were generally consistent among models. Including body mass index for all sampling years did not improve the model (data not shown). Additional models that specified interactions or non-linear effects (as product or cubic terms, respectively) were not possible (they did not converge).

Figure 4 denotes selected plots of a graphical examination of age–period–cohort effects. The period effect is apparent as decreasing PCB-153 concentrations across the sampling years and as subjects aged (Figure 4A and B). Although differences in PCB-153 concentrations among birth cohort quartiles across sampling years were not significant for some sampling years (Figure 4A), the most recently born cohort (1941–1950) appeared to have the lowest concentrations, and the earliest cohort (1925–1934) had the highest. Within sampling years, concentrations increased with age and decreased in more recent birth cohorts (Figure 4C and Supplemental Material,

14

Figure S3C). Finally, within age groups, concentrations decreased according to sampling period and birth year (Supplemental Material, Figure S3A and B, respectively). Overall, the graphical examination suggests clear period effects and additional birth cohort effects, while age effects appear relatively weak. APC patterns based on predicted PCB-153 concentrations (Figure 4D-F and Supplemental Material, Figure S3D-F) were generally consistent with patterns based on measured values.

Discussion

Intraindividual changes in POP concentrations from 1979 to 2007

Overall, our findings suggest that POP concentrations decreased during 1979-2007 in men from Northern Norway. Average summed POP concentrations in 2007 were one third of concentrations measured in 1979. The majority of POPs concentrations declined from 1979, though median concentrations of some compounds peaked in 1986 (e.g., PCB 170, 180, and 194). Peak PCB-153 concentrations were measured in 1979 and 1986 confirming this period as the years of highest human exposure, a feature which was also reproduced by CoZMoMAN (Supplemental Material, Figure S1). Large individual variability during these years was also observed, which could be expected.

The downward trends in serum concentrations likely reflect declining environmental concentrations due to reduced emissions during the same time period. This is in accordance with previous findings for environmental and human POP concentrations in Europe (AMAP 1998; Bignert et al. 1998). Our findings indicate that serum concentrations of DDTs peaked before PCBs, which is consistent with emission estimates for DDTs (Li and Macdonald 2005) and PCBs (Breivik et al. 2010). The delay in global emissions of PCBs could be due to the long

15

lifetime of PCB-containing products (e.g. transformers, capacitors) (Breivik et al. 2007). The declines of many POPs, especially *p,p'*-DDT, were exponential and indicated non-linear rates of decrease across the study period. The concentrations of some compounds were unchanged or decreased only slightly (chlordanes and Mirex), emphasizing the need to include these compounds in monitoring studies. Correlations of POPs in any pair of consecutive sampling years became stronger during the study period, possibly due to reduced concentrations and reduced variability of exposures over time.

Our results suggest that regulatory measures to reduce the manufacture and use of POPs during the 1970s and 1980s had rapid impacts not only on environmental concentrations (AMAP 1998), but also on human exposures. Substantial reductions in human intake rates relative to elimination rates are suggested.

PCB-153 concentrations and age-period-cohort effects

Time period appeared to have the strongest influence on PCB-153 concentrations based on both the graphical examination and the mixed effect analyses. Although the range of birth years (1925-1950) was relatively narrow, the mixed effect analyses also suggested an additional influence of birth cohort. This may reflect differences in cumulative exposure and dietary patterns among birth cohorts, consistent with associations between PCB-153 and age or birth cohort that have been reported based on cross-sectional studies (Bjerregaard et al. 2001; Hardell et al. 2010; Perry et al. 2005; Rylander et al. 1997; Wolff et al. 2005, Wolff et al. 2007).

Changes in dietary intakes (Quinn et al. 2012) and body mass (Wolff et al. 2007) must also be considered when evaluating time trends in human POP concentrations. When model simulations assumed higher fish consumption in earlier birth cohorts (in accordance with the available

16

dietary information and intergenerational dietary differences in Quinn et al. 2012), we observed consistent patterns of predicted and observed PCB-153 concentrations according to birth cohort (Figures 4A and 4D). Although predictions were not based on individual dietary information, CoZMoMAN model estimates were consistent with observed birth cohort patterns. This supports the use of mechanistic modeling in hypothesis testing and illustrates that an understanding of temporal trends in emissions and of confounded time factors is relevant for POP monitoring studies.

Changes in relative concentrations of POPs

The relative concentrations of POPs in human serum changed over time, both between and within POP groups. The majority of POPs evaluated were highly correlated with PCB-153 concentrations during all sampling years, which supports the use of PCB-153 as a marker compound for many legacy POPs, though it is important to note that correlations with PCB-153 concentrations weakened over time.

Proportions relative to the sum of all POPs decreased for p,p'-DDE (from 37% in 1979 to 21% in 2007) and increased for the sum of PCB 138, 153, and 180 (from 30% in 1979 to 44% in 2007). A steeper decline in concentrations for p,p'-DDE compared to PCB-153 has been reported previously based on human longitudinal studies (Hagmar et al. 2006; Hovinga et al. 1992; Vo et al. 2008). Since the p,p'-DDE/p,p'-DDT ratio is sensitive to recent exposure to p,p'-DDT (Anda et al. 2007), its increase from 12 in 1979 to 55 in 2007 suggests that exposure to p,p'-DDT was markedly reduced. The relative contribution of PCBs (and especially higher chlorinated congeners) to summed POPs increased, and thus might be expected to dominate future organochlorine POP burdens. Overall, the relative changes in POPs concentrations may be

explained by differences in emission histories, environmental persistence, exposure sources, and elimination rates.

Comparisons to other longitudinal studies

Clearly, age distribution and time of sampling relative to historic POP emissions must be considered when comparing longitudinal POP trends. The concentrations and temporal changes in PCB-153, *p,p'*-DDE, and HCB in our study population were similar to findings for younger Swedish men sampled in 1991 and 2001 (Hagmar et al. 2006). Measured concentrations were lower and peaked later in our study population than POPs concentrations measured in two longitudinal cohorts in the Great Lakes area (Hovinga et al. 1992; sampled in 1982 and 1989; Tee et al. 2003; sampled in 1980, 1990 and 1994). These similarities and differences may reflect geographical differences in environmental exposures during the same time period, in addition to different dietary patterns.

Evaluation of time-variant model predictions

In order to evaluate the performance of the CoZMoMAN mechanistic model, it is essential to challenge it with observations. Overall, its predictions were in reasonable agreement with temporal changes in median measured concentrations during 1979 to 2007, especially for PCB-153. The observed overestimation of PCB-118 by the CoZMoMAN model has also been reported by Czub and McLachlan (2004), who suggested that it could be due to an incorrect assumption of zero metabolism of this congener in humans. The co-elution of PCB-138 and PCB-163 in chromatograms is likely the reason for the apparent underestimation of PCB-138. The model did predict an initial increase of PCB-180 during the early 1980s consistent with our empirical results, although the observed decline afterwards was slightly steeper than predicted.

Study limitations

Although statistical approaches to APC effects have been much discussed in the literature, no consensus has been reached (Glenn 2003). Currently, suitable p-values in mixed effect models cannot be calculated in the statistical software. Potential interactions between the time factors could not be considered in the mixed effect model analysis, and our ability to examine APC effects was limited by small numbers of men in some age groups

Individual dietary information was not available for the model parameterization, thus, estimates of average and high fish consumption in Norwegian populations were used to reflect the potential range of fish consumption in the study population instead.

Conclusions

Longitudinal declines in legacy POPs in serum samples from Norwegian men during 1979-2007 are consistent with reduced environmental exposures in this period. Our adaptation of methods to assess APC effects based on biomonitoring data is novel and suggests that calendar time (i.e., period effects) had a major influence on observed concentrations, though birth cohort differences were also indicated. Predicted concentrations and time trends for PCB-153 were consistent with those measured. The use of age-period-cohort analysis and emission-based modeling in human biomonitoring enhances our understanding of the relationship between temporal trends in human POP burdens and historical emissions.

References

Ahacic K, Kennison RF, Kåreholt I. 2012. Changes in sobriety in the Swedish population over three decades: age, period or cohort effects? Addiction 107:748-755.

Akins JR, Waldrep K, Bernert Jr JT. 1989. The estimation of total serum lipids by a completely enzymatic 'summation' method. Clin Chim Acta 184(3):219-226.

AMAP. 1998. AMAP Assessment Report: Arctic Pollution Issues. Arctic Monitoring and Assessment Programme (AMAP) Oslo, Norway (xii+859 pp.).

AMAP. 2004. AMAP Assessment 2002: Persistent organic pollutants in the Arctic. (Arctic Monitoring and Assessment Programme (AMAP)). Oslo, Norway.

Anda EE, Nieboer E, Dudarev A, Sandanger T, Odland J. 2007. Intra-and intercompartmental associations between levels of organochlorines in maternal plasma, cord plasma and breast milk, and lead and cadmium in whole blood, for indigenous peoples of Chukotka, Russia. J Environ Monit 9(8):884-893.

Bignert A, Olsson M, Persson W, Jensen S, Zakrisson S, Litzén K, et al. 1998. Temporal trends of organochlorines in Northern Europe, 1967–1995. Relation to global fractionation, leakage from sediments and international measures. Environ Pollut 99(2):177-198.

Bjerregaard P, Dewailly E, Ayotte P, Pars T, Ferron L, Mulvad G. 2001. Exposure of Inuit in Greenland to organochlorines through the marine diet. J Toxicol Environ Health A 62:69-81.

Breivik K, Czub G, McLachlan MS, Wania F. 2010. Towards an understanding of the link between environmental emissions and human body burdens of PCBs using CoZMoMAN. Environ Int 36(1):85-91.

Breivik K, Sweetman A, Pacyna JM, Jones KC. 2007. Towards a global historical emission inventory for selected PCB congeners — A mass balance approach: 3. An update. Sci Total Environ 377(2–3):296-307.

Czub G, McLachlan MS. 2004. A food chain model to predict the levels of lipophilic organic contaminants in humans. Environ Toxicol Chem 23(10): 2356-2366.

Ding J, Kritchevsky SB, Newman AB, Taaffe DR, Nicklas BJ, Visser M, et al. 2007. Effects of birth cohort and age on body composition in a sample of community-based elderly. Am J Clin Nutr 85(2):405-410.

Glenn ND. 2003. Distinguishing age, period, and cohort effects. In: Handbook of the life course, (Mortimer JT, Shanahan MJ, eds). New York, USA:Kluwer Academic/Plenum Publishers, 465-476.

Hagmar L, Wallin E, Vessby B, Jönsson BAG, Bergman Å, Rylander L. 2006. Intra-individual variations and time trends 1991-2001 in human serum levels of PCB, DDE and hexachlorobenzene. Chemosphere 64(9):1507-1513.

Hansen S, Nieboer E, Odland JØ, Wilsgaard T, Veyhe AS, Sandanger TM. 2010. Levels of organochlorines and lipids across pregnancy, delivery and postpartum periods in women from Northern Norway. J Environ Monit 12:2128-2137.

Hardell E, Carlberg M, Nordström M, van Bavel B. 2010. Time trends of persistent organic pollutants in Sweden during 1993-2007 and relation to age, gender, body mass index, breast-feeding and parity. Sci Total Environ 408(20):4412-4419.

Helsel RD. 2005. Nondetects and Data Analysis. Statistics for Censored Environmental Data. Hoboken, NJ, USA: Wiley Interscience.

Holford TR. 1991. Understanding the effects of age, period, and cohort on incidence and mortality rates. Annu Rev Public Health 12(1):425-457.

Hovinga ME, Sowers MF, Humphrey HEB. 1992. Historical changes in serum PCB and DDT levels in an environmentally-exposed cohort. Arch Environ Contam Toxicol 22(4):362- 366.

Høyer AP, Jørgensen T, Grandjean P, Hartvig HB. 2000. Repeated measurements of organochlorine exposure and breast cancer risk (Denmark). Cancer Causes Control 11(2): 177-184.

Hung H, Kallenborn R, Breivik K, Su Y, Brorström-Lundén E, Olafsdottir K, et al. 2010. Atmospheric monitoring of organic pollutants in the Arctic under the Arctic Monitoring and Assessment Programme (AMAP): 1993-2006. Sci Total Environ 408(15):2854-2873.

Jacobsen BK, Eggen AE, Mathiesen EB, Wilsgaard T, Njølstad I. 2012. Cohort profile: The Tromsø Study. Int J Epidemiol 41(4):961-967.

Krieger N, Wolff MS, Hiatt RA, Rivera M, Vogelman J, Orentreich N. 1994. Breast cancer and serum organochlorines: a prospective study among white, black, and Asian women. J Natl Cancer Inst 86(8):589-599.

Li Y, Macdonald R. 2005. Sources and pathways of selected organochlorine pesticides to the Arctic and the effect of pathway divergence on HCH trends in biota: a review. Sci Total Environ 342(1):87-106.

Palmore E. 1978. When can age, period, and cohort be separated? Soc Forces 57(1):282-295.

Perry MJ, Ouyang F, Korrick S, Venners SA, Altshul L, Xu X, et al. 2005. Body mass index and serum 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane in nulliparous Chinese women. Cancer Epidemiol Biomarkers Prev 14(10):2433-2438.

Pitarch E, Medina C, Portolés T, López FJ, Hernández F. 2007. Determination of priority organic micro-pollutants in water by gas chromatography coupled to triple quadrupole mass spectrometry. Anal Chim Acta 583(2):246-258.

Quinn CL, Armitage JM, Breivik K, Wania F. 2012. A methodology for evaluating the influence of diets and intergenerational dietary transitions on historic and future human exposure to persistent organic pollutants in the Arctic. Environ Int 49:83-91.

Quinn CL, Wania F. 2012. Understanding Differences in the Body Burden-Age Relationships of Bioaccumulating Contaminants based on Population Cross-sections versus Individuals. Environ Health Perspect 120(4):554-559.

Quinn CL, Wania F, Czub G, Breivik K. 2011. Investigating intergenerational differences in human PCB exposure due to variable emissions and reproductive behaviors. Environ Health Perspect 119(5):641-646.

Rigét F, Bignert A, Braune B, Stow J, Wilson S. 2010. Temporal trends of legacy POPs in Arctic biota, an update. Sci Total Environ 408(15):2874-2884.

Rylander L, Dyremark E, Strömberg U, Östman C, Hagmar L. 1997. The impact of age, lactation and dietary habits on PCB in plasma in Swedish women. Sci Total Environ 207(1):55-61.

Sandanger TM, Brustad M, Lund E, Burkow IC. 2003. Change in levels of persistent organic pollutants in human plasma after consumption of a traditional northern Norwegian fish dish—Mølje (cod, cod liver, cod liver oil and hard roe). J Environ Monit 5(1):160-165.

Sandanger TM, Sinotte M, Dumas P, Marchand M, Sandau CD, Pereg D, et al. 2007. Plasma concentrations of selected organobromine compounds and polychlorinated biphenyls in postmenopausal women of Quebec, Canada. Environ Health Perspect 115(10):1429-1434.

Tee PG, Sweeney AM, Symanski E, Gardiner JC, Gasior DM, Schantz SL. 2003. A longitudinal examination of factors related to changes in serum polychlorinated biphenyl levels. Environ Health Perspect 111(5):702-707.

Vo TT, Gladen BC, Cooper GS, Baird DD, Daniels JL, Gammon MD, et al. 2008. Dichlorodiphenyldichloroethane and polychlorinated biphenyls: intraindividual changes, correlations, and predictors in healthy women from the southeastern United States. Cancer Epidemiol Biomarkers Prev 17(10):2729-2736.

Wolff MS, Anderson HA, Britton JA, Rothman N. 2007. Pharmacokinetic variability and modern epidemiology—the example of dichlorodiphenyltrichloroethane, body mass index, and birth cohort. Cancer Epidemiol Biomarkers Prev 16(10):1925-1930.

Wolff MS, Britton JA, Teitelbaum SL, Eng S, Deych E, Ireland K, et al. 2005. Improving organochlorine biomarker models for cancer research. Cancer Epidemiol Biomarkers Prev 14(9):2224-2236.

Table 1: Descriptive age statistics of study participants (N = 51, 51, 45, 48, and 52 in 1979, 1986, 1994, 2001 and 2007, respectively; all male).

	Year of measurement				
Age	1979	1986	1994	2001	2007
Median	43	50	58	65	71
Min	29	36	44	51	57
Max	54	61	69	76	82

Table 2: Mixed effect model estimates[a] (coefficients and 95% CI) of changes in PCB-153 concentrations (ng/g lipid) during 1979 to 2007 among 53 men from Northern Norway, with age, calendar period, and birth cohort as predictors.

Predictors and coefficients	Model 1: Period only (fixed effects)	Model 2: Period and age (fixed effects)	Model 3: Period and age (fixed effects) plus birth cohort (random effect)[b]	Model 4: Period and birth cohort (fixed effects)	Model 5: Period and birth cohort (fixed effects) plus age (random effect)[b]
Period[c]					
1979	Ref	Ref	Ref	Ref	Ref
1986	51 (-68, 221)	72 (-69, 284)	82 (-71, 311)	67 (-98, 326)	22 (-104, 202)
1994	-85 (-156, 12)	-66 (-159, 73)	-82 (-175, 54)	-82 (-179, 62)	-117 (-191, -16)
2001	-95 (-163, -2)	-76 (-172, 74)	-93 (-189, 52)	-84 (-181, 57)	-119 (-194, -17)
2007	-160 (-210, -90)	-150 (-224, -30)	-175 (-245, -65)	-163 (-238, -52)	-190 (-251, -103)
Age[d]					
29-47	-	Ref	Ref	-	-
47-57	-	-32 (-122, 94)	-31 (-122, 94)	-	-
57-66	-	-35 (-138, 117)	-27 (-133, 126)	-	-
66-82	-	-24 (-144, 167)	-4 (-129, 184)	-	-
Birth cohort[e]					
1925-1934	-	-	-	Ref	Ref
1934-1936	-	-	-	-73 (-210, 170)	-61 (-199, 173)
1936-1941	-	-	-	-24 (-180, 249)	-17 (-171, 244)
1941-1950	-	-	-	-139 (-246, 50)	-149 (-251, 26)
AIC[f]	154	157	176	151	137

[a]Coefficients are back-transformed from log-estimates of fixed effect variables and are in units of ng/g lipid. All models included a subject-specific random term and a random slope for sampling year. Age and birth cohort variables were divided into quartiles.

[b]Variables were added to models as random terms to allow for random variation in individuals.

[c]Coefficients express change for PCB-153 concentrations in ng/g lipid across sampling years with 1979 as the reference category.

[d]Coefficients express change in PCB-153 concentrations in ng/g lipid across age quartiles with the youngest (29-47) as the reference category.

[e]Coefficients express change in PCB-153 concentrations in ng/g lipid across birth cohort quartiles with the oldest (1925-1934) as the reference category.

[f]Akaike's information criterion. Lower numbers indicate better model fit when comparing models.

Figure legends

Figure 1: Concentrations (ng/g lipid, $\log_e$ scale) of selected POPs analyzed in repeated serum samples of men (N = 51, 51, 45, 48 and 52 in 1979, 1986, 1994, 2001 and 2007, respectively) from Northern Norway. Parlar 50 represents toxaphenes, and t-Nonachlor the chlordanes. Boxes extend from the 25th to the 75th percentile, horizontal bars represent the median, whiskers extend 1.5 times the length of the interquartile range (IQR) above and below the 75th and 25th percentiles, respectively, and outliers are represented as points. *= $p<0.05$ and **= $p<0.001$ for comparisons between pairs of consecutive sampling years.

Figure 2: Individual trend lines for PCB-153 serum concentrations measured in 1979, 1986, 1994, 2001, and 2007 in 53 men from Northern Norway, according to birth year quartile.

Figure 3: Relative contributions of individual POPs that accounted for > 1% of summed POPs in 1979 (expressed as percent of summed concentrations) in serum from men (N = 51, 51, 45, 48, and 52 in 1979, 1986, 1994, 2001, and 2007, respectively) in Northern Norway.

Figure 4: APC plots showing measured (A-C) and predicted serum PCB-153 concentrations (D-F, using the CoZMoMAN model assuming higher fish consumption in earlier birth cohorts). A and D depict longitudinal variation among birth cohorts according to sampling period, B and E longitudinal variation among birth cohorts according to age group, and C and F cross-sectional variation among sampling periods according to age group. Data points indicate ng/g lipid-adjusted concentrations (median concentrations are displayed in A-C). Differences between birth cohorts in A were significant in 1986 and 2007 (Kruskal-Wallis rank sum test, $p<0.05$).
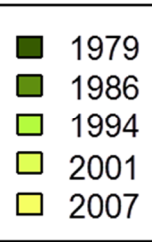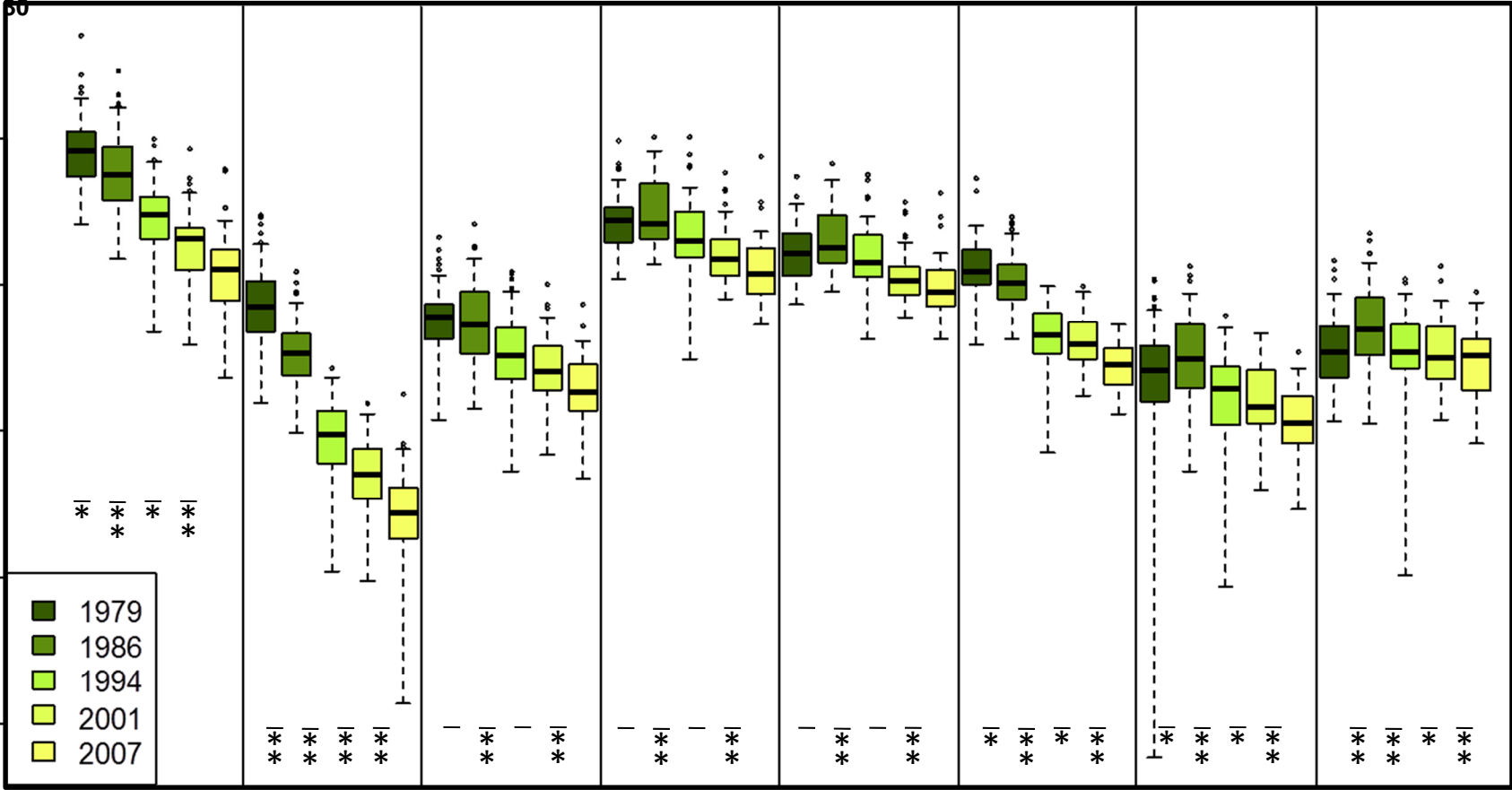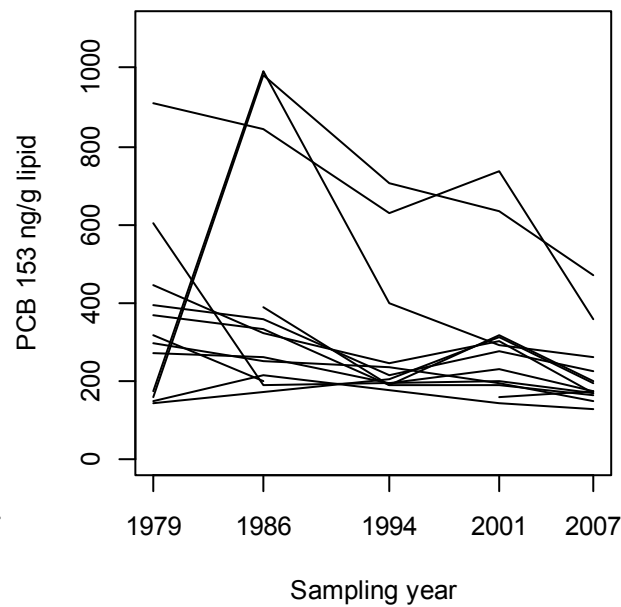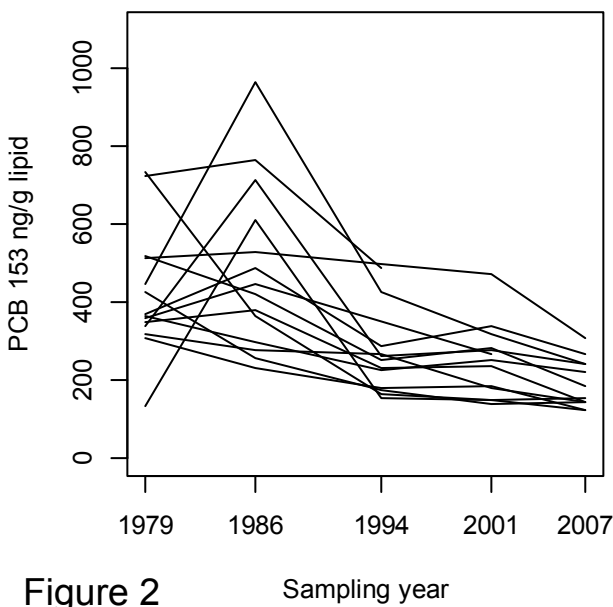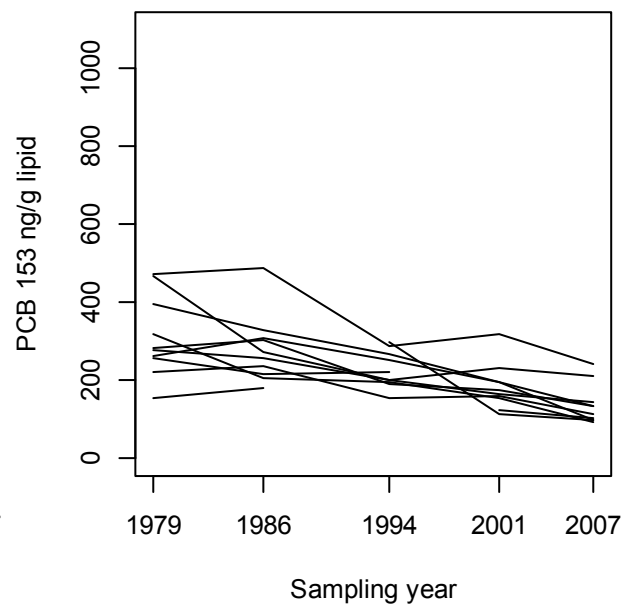
Figure 1

Birth cohort: 1925-1934
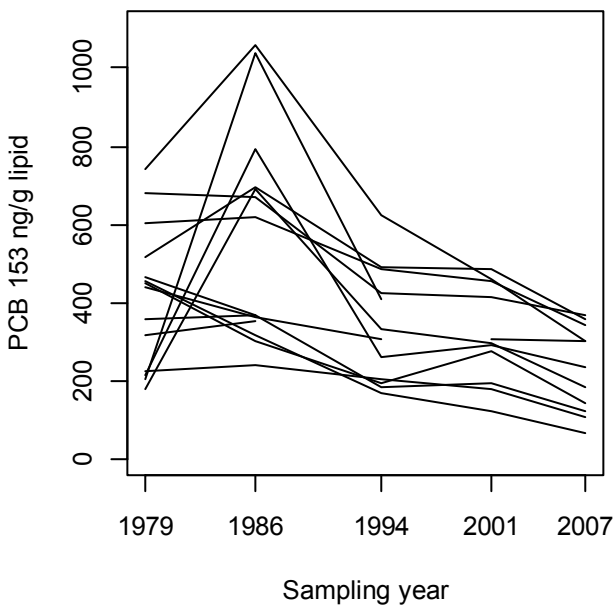
Birth cohort: 1934-1936

Birth cohort: 1936-1941
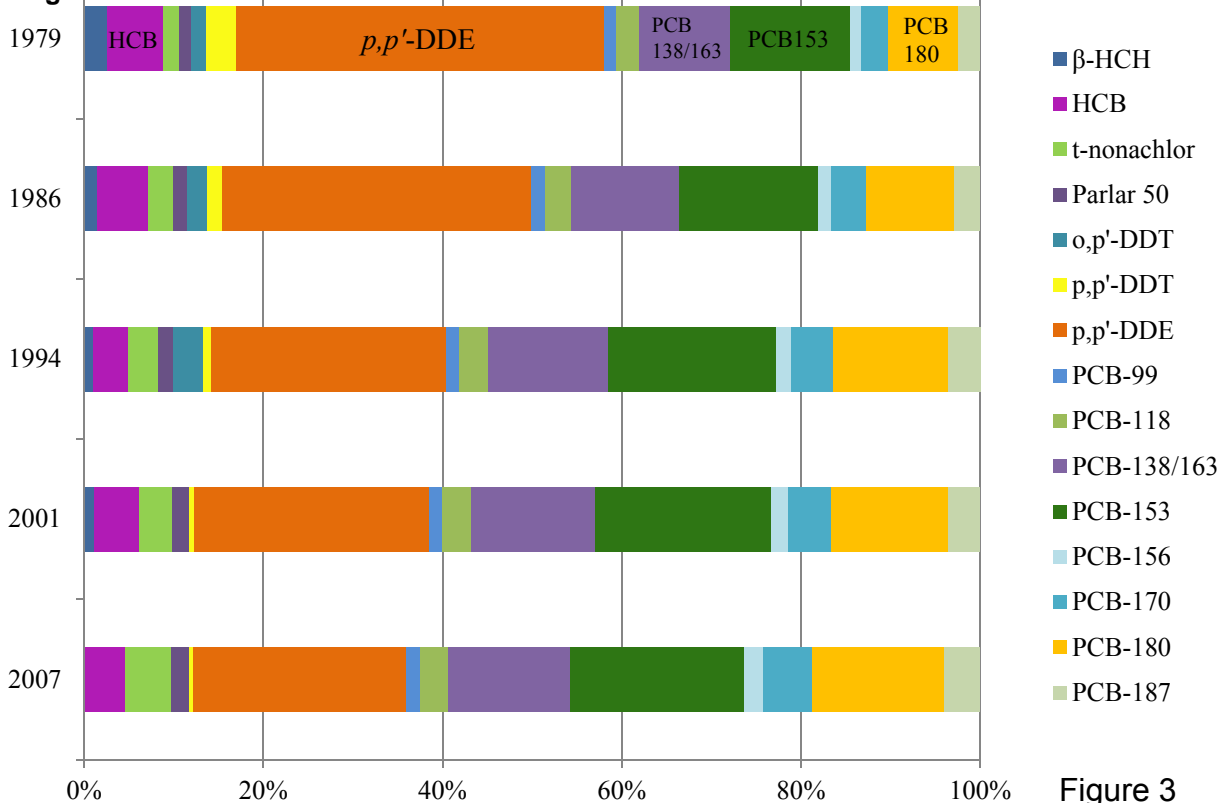
Birth cohort: 1941-1950

Figure 2

Figure 3

A) Observed results: Longitudinal organization

Birth year quartile:
- Q1: 1925-1934
- Q2: 1934-1936
- Q3: 1936-1941
- Q4: 1941-1950

B) Observed results: Longitudinal organization

Birth year quartile:
- Q1: 1925-1934
- Q2: 1934-1936
- Q3: 1936-1941
- Q4: 1941-1950

C) Observed results: Cross-sectional organization

Sampling year:
- 1979
- 1986
- 1994
- 2001
- 2007

D) Modeled results: Longitudinal organization

Birth year:
- 1930
- 1935
- 1940
- 1945

E) Modeled results: Longitudinal organization

Birth year:
- 1930
- 1935
- 1940
- 1945

F) Modeled results: Cross-sectional organization

Sampling year:
- 1979
- 1986
- 1994
- 2001
- 2007

Figure 4